METHOD FOR IMMOBILIZING ENZYMES AND MICROBIAL CELLS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending applications Ser. No. 700,562 and Ser. No. 700,577, both filed on June 28, 1976 respectively, and both now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to a method for immobilizing enzymes or microbial cells. More particularly, the invention relates to a method for preparing immobilized enzymes or microbial cells which can be easily shaped and prepared by using hydrophilic photo-curable resin having two or more photopolymerizable ethylenically unsaturated groups per molecule.

In order to minimize the instability of enzyme activity and to facilitate continuous enzymatic reaction processes, the technique of immobilizing enzymes and using them as a solid catalyst has been recently developed in several industrial fields.

As methods for preparing the immobilized enzyme, there are an adsorption method, covalent bond method, cross-linking method and entrapping method. In the last-mentioned entrapping method, the enzyme itself is not bound to any matrix but it is entrapped or micro-encapsulated in fine lattice of gel. Therefore, the activity of entrapped enzyme can be well maintained and thus various kinds of enzymes and microbial cells are treated by this method. In order to put this method into practive, however, it is necessary to select a suitable immobilizing material which entraps enzymes or microbial cells therein without causing release and has selective permeability to the substrate.

In the conventional entrapping method, an aqueous suspension of enzymes or microbial cells is first well mixed with low molecular weight hydrophilic monomers such as acrylamide, hydroxyethyl acrylate, hydroxyethyl methacrylate, hydroxypropyl acrylate and hydroxypropyl methacrylate, and the mixture is then immobilized as it stands by polymerization. However, in this method, it is difficult to properly control the selective permeability of the obtained polymer matrix, so that the entrapped enzymes or microbial cells are liable to be released from the polymer matrix. In addition, toxicity of the immobilized product is apprehended when it is used in the food industry and the pharmaceutical industry because unreacted low molecular weight monomers remain in the reaction product. Further, there is also well known an entrapping method (for example, U.S. Pat. No. 3,860,490) which comprises mixing the micro-organisms with an aqueous or organic solution of a polymer and then cross-linking the polymeric matrix to entrap the micro-organisms. In this method using the polymer solution, the toxicity problem can be settled. However, it is difficult to properly control the selective permeability of the polymer matrix.

BRIEF SUMMARY OF THE INVENTION

It is accordingly the primary object of the present invention to provide a novel method for preparing improved immobilized enzymes or microbial cells having various advantageous features.

Another object of the present invention is to provide immobilized enzymes or microbial cells which are useful and economical from an industrial view point since the activity of enzyme can be made stable and the entrapped enzymes or microbial cells are well retained in the polymer matrix.

A further object of the present invention is to provide novel immobilized enzymes or microbial cells which can be shaped into any desired configurations and has no toxicity.

The inventors of the present invention have found that the drawbacks of the prior art process, namely the toxicity problem and the control of selective permeability problem, can be eliminated if an aqueous suspension of the enzymes or microbial cells is mixed with the following hydrophilic resin and then polymerized by actinic ray irradiation. It was further found that it was possible to uniformly control the selective permeability of the resin matrix when the photopolymerization is effected only when the resin had two or more photopolymerizable ethylenically unsaturated groups per molecule.

According to the method of the present invention, an aqueous suspension of enzymes or microbial cells and hydrophilic resin is mixed well and formed into a desired shape, and then it is polymerized by irradiating actinic rays of 2500 to 6000 Å in wave length. The hydrophilic resin is characterized by having a number average molecular weight of 800 to 100,000, preferably 1000 to 70,000, by having two or more of photopolymerizable ethylenically unsaturated groups per molecule and by having hydrophilic groups.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, the molecular weight of the photo-curable resin is previously adjusted so as to pass substrates but not to release entrapped enzymes or microbial cells, and the photo-curable resin is cured by irradiating actinic rays for a short period of time in a single step operation to produce a mechanically stable immobilized product. It is desired that the photo-curable resin has hydrophilic groups to the extent that the resin uniformly mixes with the aqueous solution such as buffer solution containing the suspension of enzymes or microbial cells when they are immobilized. The activity of enzymes or microbial cells can be thus maintained in a most stable condition and the enzymes or microbial cells are immobilized without the loss of their activity. Further, in contrast to the methods employing gamma rays or electron beams, degradation of the activity of enzymes or microbial cells is not caused to occur during the curing step since the above defined actinic rays are used in the method of this invention.

In order to immobilize enzymes or microbial cells, two or more photopolymerizable ethylenically unsaturated groups are necessary for each molecule of the photo-curable resin. Further, when the number average molecular weight of the photo-curable resin is lower than 800, the cured product is liable to become brittle because the linearity of cross-linkage is low, and on the other hand, when the number average molecular weight of the photo-curable resin is higher than 100,000, the viscosity of the mixture of photo-curable resin and the suspension of enzymes or microbial cells becomes too high and the workability of resin is impaired. Therefore, the number average molecular weight of the